CLINICAL STUDY

Testosterone substitution of hypogonadal men prevents the age-dependent increases in body mass index, body fat and leptin seen in healthy ageing men: results of a cross-sectional study

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Abstract

Introduction: In healthy men, body weight and total fat content increase with advancing age, while serum testosterone levels decrease. In order to elucidate whether a causal relationship between these phenomena exists, we investigated the influence of testosterone or human chorionic gonadotrophin substitution on body mass index (BMI), total fat mass and serum leptin in testosterone-treated and untreated hypogonadal patients in comparison with ageing eugonadal men.

Methods: In a cross-sectional study, the inter-relationships of body weight, total fat mass, serum sex hormones and leptin were analysed in untreated hypogonadal men ($n = 24$; age 19–65 years), treated hypogonadal men ($n = 61$; age 20–67 years) and healthy eugonadal men ($n = 60$; age 24–78 years). Total fat mass was assessed by bioimpedance measurement. Univariate and multiple linear regression analysis was used to detect possible differences.

Results: In eugonadal men, serum testosterone levels decreased with advancing age (correlation coefficients: $r = -0.71$; $P < 0.0001$), while BMI ($r = 0.39$; $P = 0.002$), total fat content ($r = 0.51$; $P < 0.0001$) and leptin ($r = 0.48$; $P < 0.0001$) increased significantly. In untreated hypogonadal patients, an increase in BMI ($r = 0.50$; $P = 0.013$) and total fat mass ($r = 0.41$; $P = 0.044$) was also observed with advancing age. However, in substituted hypogonadal patients, no age-dependent change in BMI ($r = 0.067$; $P = 0.606$), body fat content ($r = -0.083$; $P = 0.522$), serum testosterone ($r = -0.071$; $P = 0.59$) or serum leptin ($r = -0.23$; $P = 0.176$) was found.

Conclusion: Since testosterone-substituted older hypogonadal men show BMI and fat mass similar to those of younger eugonadal men and since non-treated hypogonadal men are similar to normal ageing men, testosterone appears to be an important factor contributing to these changes. Thus ageing men should benefit from testosterone substitution as far as body composition is concerned.

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Introduction

The endocrine changes in menopausal females and the resulting influences on general well-being and health of ageing women are well established and sex hormone replacement therapy is generally recommended for postmenopausal women. In contrast, the state of knowledge about endocrine changes in men lags behind that of women and the possible benefits of androgen replacement in ageing men are not fully established. Ageing in healthy men is accompanied by a continuously slow, rather than abrupt, decrease in testosterone serum levels. However, there is wide inter-individual variability of serum testosterone levels at any age (1–5). With advancing age, 20–30% of men have serum testosterone levels in the hypogonadal range

while, in others, testosterone levels remain within the normal range for healthy younger men (1, 4, 6). Testosterone serum levels tend to remain higher in healthy older men compared with ageing men with different diseases (3).

In addition, several physiologic changes related to increasing age have been documented. Muscle mass, muscle strength as well as bone density decrease in ageing men and the proportion of adipose tissue increases (7). All these variables are known to be hormone dependent to some extent. However, it is unclear whether age-related changes in these anthropometric parameters are directly related to the changes in hormones and in particular to declining testosterone.

Compared with healthy age-matched men, hypogonadal men have increased body mass index (BMI)

but reduced lean BMI (8–10) and obese young men have reduced serum testosterone levels (11, 12). With both testosterone as well as growth hormone treatment these variables improve (8, 13, 14). Testosterone administration to hypogonadal men improves bone density and muscle mass and decreases adipose tissue (4, 9, 15–18). Moreover, in healthy adipose men, testosterone administration leads to a reduction in visceral fat mass and increasing muscularity (14). The reduction in testosterone serum levels in adipose men is in line with these observations.

Independent of the patient's age, the aim of testosterone substitution therapy in hypogonadal men is to achieve testosterone serum levels within the normal range (19). Therefore, in hypogonadal men on long-term testosterone substitution none of the testosterone-related physiological changes should be observed, and testosterone-treated hypogonadal men may thus serve as a model for ageing men in whom serum testosterone levels are maintained in the range of healthy young men.

We tested this model by comparing BMI, body fat content, leptin and serum testosterone in testosterone-treated and untreated hypogonadal patients and in healthy men.

Subjects and methods

Three groups comprising a total of 145 adult men were included in this prospective cross-sectional investigation. Sixty-one hypogonadal patients on testosterone or human chorionic gonadotrophin (hCG) substitution therapy (means $\pm$ S.D.; age 35.3 ± 11.6 years) formed group 1; of these, 16 men were treated with hCG (500–5000 IU twice a week; Schering, Berlin, Germany) and 45 with injectable, oral or transdermal testosterone (testosterone enanthate, 250 mg ($n = 35$; Schering), oral testosterone undecanoate, 120 mg/day ($n = 3$; Organon, Oberschleißheim, Germany) or daily transdermal testosterone patch ($n = 7$; Ferring, Kiel, Germany)). All patients received substitution therapy for at least 12 months. Twenty-two patients with primary hypogonadotropic hypogonadism (age 32.7 ± 12.0 years) and 39 patients with hypogonadism (age 36.7 ± 11.2 years; 26 patients with idiopathic hypogonadism or Kallmann's syndrome and 13 patients with hypopituitarism) were recruited for the study.

Group 2 consisted of 24 newly diagnosed hypogonadal patients without previous androgen treatment (age 37.0 ± 12.4 years; 15 with primary (age 40.9 ± 11.7 years) and 9 with secondary hypogonadism (age 30.4 ± 15 years)). The cause of hypogonadism was known in all patients in groups 1 and 2; patients attending our clinic for age-related reduced testosterone serum levels were excluded.

Group 3 was comprised of 60 unselected normal men (age 45.6 ± 16.6 years) recruited from the general

population. The younger men ($n = 31$) were volunteers for clinical studies on male contraception (20–22). None reported any previous sex hormone treatment, no concomitant medication potentially interfering with sex hormones or body composition was allowed. All measurements were performed during the screening phase of the trial to avoid a positive selection through inclusion criteria. The older men ($n = 29$) were recruited from the community by personal contacts. As with advancing age an increasing proportion of men have testosterone serum levels in the hypogonadal range, we did not exclude men with hypogonadal testosterone serum levels when no clinical evidence for the hypogonadism was seen. In all study groups, patients and/or volunteers with chronic diseases or medications which might interfere with sex hormones and body composition were excluded (e.g. patients under high-dose corticosteroid treatment or patients with a history of antineoplastic therapy). In the groups of hypogonadal men, only men with physiologic hormone replacement therapy were included. As growth hormone influences body composition and sex hormone production, hypogonadal patients on growth hormone substitution were excluded.

Morning blood samples for hormone analysis were drawn between 0800 and 1100 h. In patients treated with transdermal testosterone patches ($n = 7$) or oral testosterone undecanoate ($n = 3$), blood was taken 3–6 h after administration. In patients given intramuscular testosterone enanthate ($n = 35$), blood was preferentially collected during the second week after application. In hCG-treated patients ($n = 16$), blood was obtained 2 days after injection.

Testosterone was determined by highly specific solid-phase, two-site fluoroimmunoassay (AutoDELFIA Testosterone; Wallac ADL-GmbH, Freiburg, Germany). The detection limit was 0.469 nmol/l. The intra- and interassay coefficients of variation were 5.8% and 10.8% respectively. Oestradiol was measured by solid-phase immunofluoroassay (AutoDELFIA Estradiol; Wallac ADL-GmbH). The detection limit for oestradiol was 12.7 pmol/l. The intra- and interassay coefficients of variation for oestradiol were 6.6% and 8.1% respectively. Sex hormone-binding globulin (SHBG) was determined by a specific fluoroimmunoassay (DELFIA SHBG; Wallac ADL-GmbH). The intra- and interassay coefficients of variation for SHBG were 4.9% and 7.2% respectively. The normal range for SHBG is 11–71 nmol/l. Serum levels of leptin were measured by radioimmunoassay (Linco Research Inc., St Charles, MO, USA). The detection limit was 0.5 μ g/l. The intra- and interassay coefficients of variation for leptin were 5.2 and 6.0% respectively. Bioactive unbound testosterone was calculated according to the method established by Vermeulen *et al.* (23).

Percent body fat mass was assessed by bioelectrical impedance using a multiple frequency bioimpedance analyser (Data Input, Frankfurt/Main, Germany) with

four cutaneous electrodes attached to specific sites of the dorsal surface of the hand and anterior surface of the ipsilateral foot. Bioelectrical impedance is based upon the conductivity of the human body, which depends on the relationship between fat and lean tissue. For total fat mass, the intra-individual coefficient of variance based on 13 repeated measurements by one single clinician was 2.3% and the inter-individual coefficient of variance based on duplicated measurement of adipose ($n = 3$) and normal ($n = 4$) volunteers by two independent clinicians was 6.1%. BMI was calculated by dividing body weight (kg) by square height (m).

Statistical analysis

Normal distribution of all hormone variables was tested by the Kolmogorov–Smirnov test and logarithmic transformation was performed when applicable. Bivariate correlation analysis was performed using age, anthropometric data and the various hormonal parameters. Two-sided $P < 0.05$ was considered significant.

Multiple regression analysis was applied for evaluation of joint effects of age, sex hormones, SHBG and leptin on BMI and body fat mass. Due to the small sample size, multiple regression analysis was not performed in group 2. Computations were performed using the statistical software package SPSS version 10.0 for Windows (Chicago, IL, USA). Unless otherwise stated, results are given as means $\pm$ S.D.

Results

In group 1, no significant differences were found between patients with primary or secondary hypogonadism for any of the investigated variables; therefore, the two subgroups were combined to increase statistical power. In group 1, no significant changes in BMI or body fat content were observed with advanced age and no age-related changes in serum testosterone levels were seen (Fig. 1). Oestradiol and SHBG serum levels also remained unchanged (Table 1). No decline in leptin serum levels was seen with advanced age; a strong association between leptin serum levels and BMI, body fat content, bioactive testosterone and SHBG was noted.

In the untreated hypogonadal men (group 2), an increase in body fat content, BMI and leptin was observed with increasing age (Table 2). Leptin serum levels were positively associated with BMI and body fat content. No associations between sex hormones or SHBG and anthropometric data were noted.

In healthy men (group 3), a significant increase in BMI and body fat content was observed with advanced age. Serum testosterone levels decreased with advanced age ($r = -0.71$; $P < 0.001$). Increased serum leptin and SHBG levels were found in men of advanced age

Figure 1 Age dependency of BMI, percentage of body fat mass and serum testosterone levels in healthy men (squares; $n = 60$), hypogonadal men under adequate testosterone substitution therapy (circles; $n = 61$) and untreated hypogonadal men (triangles; $n = 24$).

(Table 3). No associations between oestradiol and age or any anthropometric variable were found.

In treated hypogonadal men and in normal volunteers (groups 1 and 3), stepwise linear regression using leptin, testosterone, SHBG, oestradiol and age as the independent variables, showed BMI and body fat content were highly significantly associated with elevated leptin levels ($P < 0.001$). Only in the group of unselected volunteers did the variable age show a

Table 1 Correlation of the investigated variables in treated hypogonadal men (group 1; $n = 61$). In the upper right area of the table the coefficients of correlation of the investigated parameters are shown, the lower left area demonstrates the corresponding levels of significance (in *italics*).

Coefficient of correlation/significance								
	Age (years)	BMI	Total fat content (%)	Testosterone (nmol/l)*	Bioactive testosterone (pmol/l)*	Oestradiol (pmol/l)*	SHBG (nmol/l)*	Leptin (μ g/l)*
Age (years)		0.067	-0.083	-0.071	-0.145	-0.039	0.189	-0.227
BMI	<i>0.606</i>		0.612	-0.115	-0.272	0.131	-0.374	0.565
Total fat content (%)	<i>0.522</i>	<i>0.0001</i>		-0.046	-0.031	-0.003	-0.136	0.617
Testosterone (nmol/l)	<i>0.587</i>	<i>0.378</i>	<i>0.725</i>		0.869	0.396	-0.133	0.228
Bioactive testosterone (pmol/l)	<i>0.264</i>	<i>0.034</i>	<i>0.812</i>	<i>0.0001</i>		0.352	-0.597	0.358
Oestradiol (pmol/l)	<i>0.768</i>	<i>0.320</i>	<i>0.980</i>	<i>0.002</i>	<i>0.006</i>		-0.077	0.120
SHBG (nmol/l)	<i>0.145</i>	<i>0.003</i>	<i>0.296</i>	<i>0.306</i>	<i>0.001</i>	<i>0.557</i>		-0.391
Leptin (μ g/l)	<i>0.176</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.174</i>	<i>0.029</i>	<i>0.485</i>	<i>0.017</i>	

* After logarithmic transformation.

slight but significant ($P = 0.034$) additional association with BMI. All sex hormones investigated were excluded from this model.

Discussion

In this cross-sectional study, we investigated age-dependent changes in BMI, body fat content and sex hormones in normal healthy men as well as androgen-substituted hypogonadal men. In normal men and even more so in untreated hypogonadal men, an increase in BMI and percentage of body fat was observed with advancing age. In addition, serum testosterone levels decreased in parallel in normal men. In contrast, in hypogonadal men under adequate replacement therapy no decrease in testosterone serum levels was observed with advancing age. A slight increase in body mass, but no significant increase in the percentage of body fat is noted in these patients.

According to the results of the multiple linear regression analysis we cannot conclude which variable, declining testosterone serum levels or age *per se*, is responsible for the increase in BMI and body fat content

in normal men, as there is a strong and highly significant interdependency between these variables. However, in hypogonadal patients receiving adequate testosterone substitution, where the regulating variable testosterone remains virtually unchanged with increasing age, no change in BMI and body fat content could be observed, indicating that the decline in sex hormones has an essential influence on the 'physiologic' increase in BMI and body fat content.

Leptin may be the main mediator of the influence of sex hormones on adipocytes: testosterone has been found to suppress leptin synthesis by adipocytes both *in vivo* and *in vitro* (24). In healthy normal men, suppression of endogenous testosterone production with gonadotrophin-releasing hormone (GnRH) agonists (25) or GnRH antagonists (26) results in an increase of leptin serum levels and, vice versa, testosterone administration to normal healthy men results in reduced leptin serum levels (25). Testosterone substitution therapy of hypogonadal men normalises elevated leptin serum levels (27).

It has been proposed that obesity in humans may be caused by resistance to leptin (28). However, the control of leptin secretion in humans is not well

Table 2 Correlation of the investigated variables in untreated hypogonadal men (group 2; $n = 24$). In the upper right area the coefficients of correlation of the investigated parameters are shown, the lower left area demonstrates the corresponding levels of significance (in *italics*).

Coefficient of correlation/significance								
	Age (years)	BMI	Total fat content (%)	Testosterone (nmol/l)*	Bioactive testosterone (pmol/l)*	Oestradiol (pmol/l)*	SHBG (nmol/l)*	Leptin (μ g/l)*
Age (years)		0.499	0.414	-0.110	-0.041	0.168	-0.145	0.596
BMI	<i>0.013</i>		0.572	0.340	0.376	0.344	-0.339	0.619
Total fat content (%)	<i>0.044</i>	<i>0.004</i>		0.079	0.092	0.262	-0.060	0.579
Testosterone (nmol/l)	<i>0.608</i>	<i>0.104</i>	<i>0.714</i>		0.964	0.492	-0.576	0.325
Bioactive testosterone (pmol/l)	<i>0.850</i>	<i>0.070</i>	<i>0.667</i>	<i>0.0001</i>		0.513	-0.770	0.389
Oestradiol (pmol/l)	<i>0.455</i>	<i>0.117</i>	<i>0.240</i>	<i>0.020</i>	<i>0.015</i>		-0.378	-0.096
SHBG (nmol/l)	<i>0.500</i>	<i>0.105</i>	<i>0.781</i>	<i>0.003</i>	<i>0.0001</i>			-0.450
Leptin (μ g/l)	<i>0.032</i>	<i>0.024</i>	<i>0.038</i>	<i>0.278</i>	<i>0.188</i>	<i>0.779</i>	<i>0.123</i>	

* After logarithmic transformation.

Table 3 Correlation of the investigated variables in normal men (group 3; $n = 60$). In the upper right area the coefficients of correlation of the investigated parameters are shown, the lower left area demonstrates the corresponding levels of significance (in italics).

	Coefficient of correlation/significance							
	Age (years)	BMI	Total fat content (%)	Testosterone (nmol/l)*	Bioactive testosterone (pmol/l)*	Oestradiol (pmol/l)*	SHBG (nmol/l)*	Leptin (μ g/l)*
Age (years)		0.393	0.510	-0.710	-0.824	0.056	0.554	0.478
BMI	<i>0.002</i>		0.595	-0.395	0.380	0.013	0.115	0.595
Total fat content (%)	<i>0.0001</i>	<i>0.0001</i>		-0.333	-0.413	-0.077	0.333	0.652
Testosterone (nmol/l)	<i>0.0001</i>	<i>0.002</i>	<i>0.010</i>		0.894	0.209	-0.229	-0.392
Bioactive testosterone (pmol/l)	<i>0.0001</i>	<i>0.003</i>	<i>0.001</i>	<i>0.0001</i>		0.105	-0.638	0.392
Oestradiol (pmol/l)	<i>0.678</i>	<i>0.923</i>	<i>0.566</i>	<i>0.116</i>	<i>0.435</i>		0.137	0.125
SHBG (nmol/l)	<i>0.0001</i>	<i>0.390</i>	<i>0.011</i>	<i>0.084</i>	<i>0.0001</i>	<i>0.309</i>		0.178
Leptin (μ g/l)	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.004</i>	<i>0.004</i>	<i>0.372</i>	<i>0.207</i>	

* After logarithmic transformation.

understood. Leptin has been proposed as the key link between energy stores and nutritional stores and the reproductive axes (29–31). Our data confirm the strong association between leptin and testosterone as well as between leptin and BMI and body fat content.

Studies on age-dependent changes of serum oestrogens give conflicting results (3, 12, 32–34). Lower testosterone levels and higher SHBG levels can intensify the influence of oestrogens on the organism, which may explain the increased incidence of gynaecomastia in senescent men (35). Our present data fail to indicate a decline of serum oestradiol levels with age in any group; moreover, no correlation between oestradiol serum levels and BMI or body fat content could be observed, indicating that oestradiol has no predominant influence on body composition in men. This is somewhat surprising, as it is generally accepted that testosterone is aromatised into oestradiol in adipose tissue and, thus, oestradiol serum levels should be elevated in adipose men. However, other studies also fail to correlate adiposity and oestradiol serum levels in men (4, 31, 36, 37).

From puberty onwards, males and females differ in bodily distribution of adipose tissue. Subcutaneous fat largely disappears in normal weight pubertal boys. From the age of 20–30 years on, men have approximately twice as much visceral fat storage as women with comparable BMI. Men preferentially store fat intra-abdominally, while premenopausal women usually store fat subcutaneously on hips and thighs. The fact that androgens are involved in this sex-specific fat distribution has become increasingly clear, although the precise mechanism remains unknown.

Administration of testosterone induces changes in body composition, namely, an increase in both bone density and lean body mass and a decrease in fatty tissue. However, the process by which body composition affects the secretion of testosterone is highly controversial. Disease states such as obesity and chronic hypercortisolism are associated with increased adiposity and/or the central distribution of fat. Ageing, charac-

terised by excess adiposity, is also associated with impaired secretion of testosterone. In these states, both spontaneous and stimulated secretion of testosterone are severely impaired. Interestingly, in women the opposite relationship between androgens and obesity is present: testosterone is positively correlated with BMI and leptin levels (38–40). Nevertheless, it is not known whether obesity causes hyperandrogenaemia or hyperandrogenaemia causes obesity in women.

Testosterone not only has anabolic effects but this steroid and its metabolites, oestradiol and dihydrotestosterone, also have psychotropic effects (41). As well as improving libido in hypogonadal men, testosterone administration also results in increased general well-being and physical/mental activity. Although no direct measurement of lean body mass was performed and we cannot exclude the possibility that the reduced body fat content was caused by increased physical activity of testosterone-treated men, our data document that testosterone substitution can be beneficial for ageing hypogonadal men, at least with regard to body composition.

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